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HPLC analysis of Polycyclic Aromatic Hydrocarbons(PAHs) in Mussels(*Mytilus edulis*) living in the Intertidal Zone of Kori, Korea

Il Noh

Department of Environmental Engineering, Korea Maritime University

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Polycyclic aromatic hydrocarbons(PAHs) are ubiquitous contaminants in coastal marine environment. PAHs enter estuarine and nearshore marine environment via several routes such as combustion of fossil fuels, domestic and industrial effluents and oil spills.

In August of 1997, mussels(*Mytilus edulis*) were collected at 6 sites near Kori nuclear power plant in order to analyze the PAH content by HPLC with uv/vis detection. Unfortunately, I could not find any living oysters in which I firstly intended to measure the PAH content in the study area.

NPTHL and ANCPL were the major dominant PAH compounds in mussels living in the intertidal zone of Kori, Korea, and DahA, BbF, BaP were the next dominant PAH group in mussels in the study area.

The mean concentrations of 15 PAH in mussels ranged from 3.2 to 1,680 ppb(mean 105±60.5 ppb).

Compared with other studies world over, the concentrations of carcinogenic PAHs were relatively low in mussels in the study area, even though total PAH content was rather high. According to N/P(Naphthalene/Phenanthrene) ratio(147) and the ratio of 2~3 ring to 3~5 ring PAHs(58~90 %) in mussels in the study area, I expect that the major source of PAHs in this study area is rather fresh petroleum-derived.

This study presents preliminary data for the PAH levels in mussels from the intertidal zone of Kori, and the data will hopefully be utilized for the assessment of oil pollution in the East Sea, Korea.

Key words :

1. Introduction

Estuarine and coastal environments are affected by various kinds of pollutants. Of all things, oil pollution has recently received the greatest public attention because of the direct damage to fisheries, and the harmful effects on marine lives.

Polycyclic or polynuclear aromatic hydrocarbons(PAHs) are a group of compounds composed of two or more fused aromatic rings. PAHs have been the focus of numerous studies in the world because they are potentially carcinogenic, mutagenic, and teratogenic to aquatic organisms and humans consuming PAH- contaminated food.

PAHs enter marine environment via several routes ; domestic and industrial effluents, oil spill, incomplete combustion of fossil fuels, forest and brush fires, terrestrial contributions and natural sources such as biosynthesis by plant and microorganisms. However, oil spill and incomplete combustion of fossil fuels are major sources of PAHs in marine environment[10,11]. They typically adsorb to fine particulate materials suspended in estuarine waters and sediment seafloor[4].

Corresponding author ; Il Noh, Department of Environmental Engineering, Korea Maritime University
Phone : +82-51-410-4327
E-mail : ilnoh@hanara.kmaritime.ac.kr

Once PAHs have been introduced to a harbor or coastal zone, they accumulate in sediments because of their hydrophobicity and partitioning to the organic carbon-coated particles[6]. Since PAHs have low solubility in water and tend to be transported with suspended sediment, most PAHs introduced into aquatic environments are accumulated in bottom sediment. PAHs, as well as other organic pollutants, remain in the sediment usually depending on their rate of degradation in sediments[3]. Mussels and oysters are intertidal and subtidal organisms, which attach themselves to various substances and filter-feed on suspension. This study tried to analyze PAH concentration in intertidal organisms(especially mussels and oysters) in the East Sea of Korea filter-feeding on suspended particles on which PAH compounds, if present, were adsorbed.

Measurement of PAHs in living organisms in the intertidal zone of East Sea can hardly be found up to the present. This study tried to develop the method of PAH measurement in intertidal organisms living in the East Sea of Korea by High Performance Liquid Chromatography(HPLC), which has been used world-wide in measurement of PAHs in marine organisms.

This study presents preliminary data on the PAH levels in mussels(and oysters, in which I firstly intended to measure PAH content, however, failed to sample) from the intertidal zone near Kori nuclear power plant in order to contribute to the establishment of database on PAH contamination in mussels in the East Sea of Korea. And this study also tried to trace the possible sources of PAHs accumulated in mussels living in the vicinity of Kori nuclear power plant.

2. Materials And Methods

2.1 Chemicals

15 unsubstituted PAHs in coastal environment near Kori nuclear power plant were investigated: Naphthalene(NPTHL), Acenaphthylene(ANCPL), Acenaphthene(ACNPN), Fluorene(FLURN), Phenanthrene(PHEN), Anthracene(ANTHR), Fluoranthene(FLRTH), Pyrene(PYR), Chrysene(CHRY), Benzo(b)fluoranthene(BbF), Benzo(k)fluoranthene(BkF), Benzo(a)pyrene (BaP), Dibenz(a,h)anthracene (DahA), Benzo(g.h.i)peryline (BghiP) and Indeno(1,2,3,-cd)pyrene (I123cdP). Of those, EPA recently reported that the following seven PAHs are typically considered as possible or probable carcinogens : Chrysene (CHRY), Benzo(b) fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Dibenz(a,h)anthracene (DahA), Benzo(g.h.i)peryline (BghiP) and Indeno (1,2,3,-cd)pyrene (I123cdP)[7].

2.2 Sampling and Extraction of PAHs from mussels(*Mytilus edulis*)

Mussels(*Mytilus edulis*) were collected at six sites by SCUBA divers from 1.5 ~ 3 m water depth near the intertidal zone of Kori nuclear power plant, Korea, during August of 1997(Fig. 1).

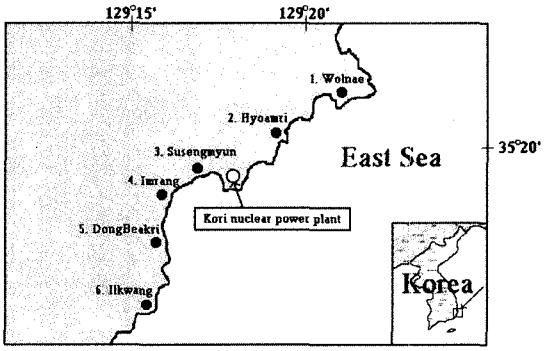

Fig. 1. Location of sampling sites in the East Sea, Korea.

Sampling sites were located in near residential area. Domestic wastewater and runoff have been discharged into this area via several streams., red tides tend to occur annually in this area In summer seasons.

The length of the mussels ranged from 3±0.11 cm to 3.8±0.08 cm, indicating they were young specimens. Samples were stored frozen under darkness in an icebox to minimize possible degradation caused by photo-oxidation or bacterial action and brought to the lab, and stored at -20 ℃ prior to analysis. Glass devices were prepared by heating at 450 ℃ to remove any possible organic contamination.

HPLC grade reagents(hexane, acetone, diethylether, petroleum ether, methanol, dimethylsulfoxide(DMSO), cyclohexane, etc.) and ultrapure distilled water were used for all extraction procedures. The shells were removed and the tissue

of mussels(20 g, wet wt) were homogenized with macerator and dried with anhydrous sodium sulfate (Na_2SO_4). The fats in thimble filter were Soxhlet-extracted with a mixture of hexane, acetone, diethylether and petroleum ether for 6 hour[11]. Then the fatty residues mixed with methanol containing 7 g of potassium hydroxide(KOH) were refluxed for 3 hours. After digestion, the solution was separated three times with a cyclohexane[12,13]. To separate PAHs from the aliphatic hydrocarbons, the liquid-liquid extraction procedure applied with dimethylsulfoxide(DMSO)[9]. The remaining portion was partitioned three times with a cyclohexane and evaporated to 1 ㎖ volume under a stream of nitrogen gas. Concentration of PAHs in mussel are expressed in units of ㎍/kg wet weight.

2.3 HPLC system

The analysis of PAHs in mussels was carried out by computer-assisted HPLC system(Linear Instrument Co.), equipped with a Model S-1100 binary solvent delivery system, a Model S-2000 automatic gradient controller and an injector fitted with a GROM-SIL 120 ODS-5 ST column(250×4 ㎜, 5 ㎛ particle size).

The flow rate of mobile phase was held constant at 0.8 ㎖/min under the condition of 0.5 bar pressure.

Solvent A of acetonitril and solvent B composed of ultrapure distilled water and acetonitril(50 : 50, v/v) were utilized as mobile phases.

A gradient solvent system for the elution of PAHs in this study was programmed as follows : delivery program of solvent was planned at 100 % solvent B for the initial condition, 70 % solvent B at 5 min, 20 % solvent B at 15 min, 10 % solvent B at 20 min, 5 % solvent B at 25 min, and then followed by isocratic held 100 % solvent A until 30 min(Table 1).

Sample was injected by using 20 ㎕ syringe. Analytical blank test was carried out between each sample run and no analytical contamination has found in the HPLC system.

The peaks of PAHs were identified and quantified using uv/vis detection setting at 254 nm. The management of chromatograms, integration and calibration of data were carried out using Peaksimple Serial Data Program system(SRI Model 202).

Table 1. Binary gradient program used in this study

Time (min)	Solvent A	Solvent B
	CH_3CN(%)	H_2O : CH_3CN(50 : 50, v/v) (%)
0	0	100
5	30	70
15	80	20
20	90	10
25	95	5
30	100	0

3. Results And Discussion

3.1 Dominant PAH compounds

The chemical formula, structure, and retention times of each compound of PAHs are summarized in Table 2.

In the present study, certified reference material was used for verification of PAHs, and each PAHs was identified on the basis of retention time marked in the chromatograms and quantified by uv/vis detector responses of the samples with the corresponding peaks of authentic standard solution (Supelco : Lot 125H0792). Separation for individual NPTHL, ANCPL, PHEN, ANTHR, FLRTH, PYR, CHRY, BaP, DahA, BghiP and I123cdP in the standard solution was satisfactory by the HPLC system, while ACNPN, FLURN, BbF and BkF were not sharply separated with uv/vis detection (Fig. 2).

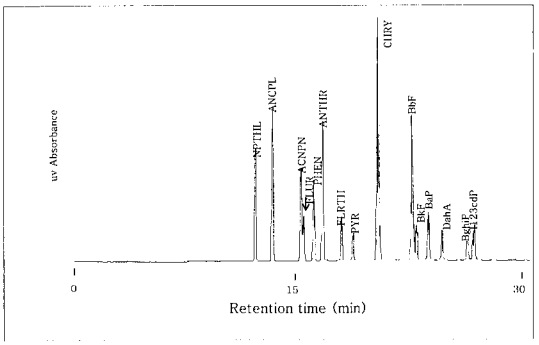

Fig. 2. Chromatogram of PAH standard solution by HPLC with uv/vis detection. BaA can't eluted at 254 nm.

The concentrations of PAHs in mussels from

Table 2. The chemical formula, structures and retention times(R*t*) of PAHs analyzed in the study
mw : molecular weight

No.	COMPOUND (ABBREV)	ALTERNATIVE NAME	FORMULAR (MW)	STRUCTURE	R*t*(min)*
1	Naphthalene(NPTHL)		$C_{10}H_8$(128)		12.16
2	Acenaphthylene(ANCPL)		$C_{12}H_8$(152)		13.28
3	Acenaphthene(ACNPN)		$C_{12}H_{10}$(154)		15.15
4	Fluorene(FLURN)		$C_{12}H_{10}$(166)		15.38
5	Phenanthrene(PHEN)		$C_{14}H_{10}$(178)		16.00
6	Anthracene(ANTHR)		$C_{14}H_{10}$(178)		16.55
7	Fluoranthene(FLRTH)		$C_{16}H_{10}$(202)		17.80
8	Pyrene(PYR)		$C_{16}H_{10}$(202)		18.55
9	Chrysene(CHRY)		$C_{18}H_{12}$(228)		20.13
10	Benzo(b)fluoranthene(BbF)	3,4 Benzfluoranthene	$C_{20}H_{12}$(252)		22.40
11	Benzo(k)fluoranthene(BkF)	11,12 Benzfluoranthene	$C_{20}H_{12}$(252)		22.73
12	Benzo(a)pyrene(BaP)	3,4 Benzopyrene	$C_{20}H_{12}$(252)		23.45
13	Dibenz(a.h)anthracene (DahA)	1,2,5,6 Dibenzanthracene	$C_{22}H_{14}$(278)		24.33
14	Benzo(g.h.i)peryline (BghiP)	1,12 benzperylene	$C_{22}H_{12}$(276)		26.06
15	Indeno(1.2.3-cd)pyrene ($I_{123\ cd}P$)	o -Phenylenepyrene	$C_{22}H_{12}$(276)		26.40

* The Retention times of PAHs analyzed were drawn from uv/vis detection.

six sites are presented in Table 3. And the representative chromatograms of PAHs of mussels analyzed by uv/vis detection at site 6 are presented in Fig. 3. The highest PAH content observed was ANCPL(5,900 ppb) in mussels at site 2 near the nuclear power plant. The second most highest PAH compound was NPTHL(81 ppb), and DahA, BbF, BaP were the next dominant PAH group in mussels in the study area. I123cdP, however, was not detected in any mussel ay all sites.

Total PAHs ranged from ranged from 119.8 to 6,351.3(mean 1,923±1,069 ppb) in mussels in the study area. The total PAHs in mussels reported in Chinhae bay, Korea[5], ranged from 498 to 2,060 ppb wet wt(mean 760 ppb). Compared the present study with Lee's result in Chinhae bay, the PAH content in mussels from Kori and Chinhae bay showed generally similar. The levels of PAHs in marine ecosystem have been well documented in other countries, but not much data are available in Korea.

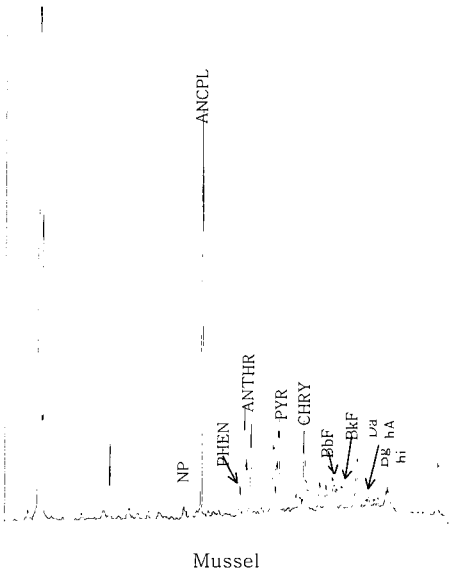

Fig. 3. Chromatogram of PAHs in mussel with uv/vis detection at site 6.

3.2 Comparison with data of foreign counties

The total PAH content in mussel in the Gulf of Naples from Italian Central Mediterranean coasts[2] varied from 2 to 60 ppb(Table 3). Most individual

Table 3. The concentration of PAHs in mussels(㎍/㎏ wet wt) from the intertidal zone of Kori.

	Site 1	Site	Site 3	Site 4	Site 5	Site 6	Mean±SE*	[a]Italy
NPTHL	22.5	85.5	334	16.5	25	2.5	81±51	ND
ANCPL	42	5,900	3,260	290	26.5	565	1,680±981	60
ACNPN	ND	ND	129	ND	ND	ND	126	35
FLURN	ND	ND	117	ND	ND	ND	65	5
PHEN	8	5.5	1.5	0.02	1.5	3	3.3±1.3	4
ANTHR	ND	31.5	17.5	3.5	10.8	11.5	15±4.7	5
FLRTH	ND	10.5	16.5	2	3	4.5	7.3±2.7	21
PYR	17.5	10.3	34.5	13	17	24	19±3.5	24
CHRY	1.5	2	1.5	ND	2.5	8.5	3.2±1.3	13
BbF	11.5	26	27	6	18.5	10	16.5±3.5	46
BkF	15.5	10.5	2.5	10.5	1.5	6	7.8±2.1	4
BaP	3	64	6	3	2.5	ND	15.7±12	5
DahA	5.5	77.5	5.5	ND	11	1.6	20.2±14.3	20
BghiP	ND	128	ND	3.1	ND	4.1	72±41	22
$I_{123cd}P$	ND	ND	ND	ND	ND	ND		
Total	127	6,351.3	3,952.5	347.6	119.8	640.7	1,923±1,069	

*SE : Standard Error, ND : Not detected
[a]Italy : Cocchieri et al.(1990)[2)]

PAH contents in the Gulf of Naples were very similar to those in mussels from Kori, Korea, except NPTHL and ANCPL(see Table 3). NPTHL was not reported in mussels in the Gulf of Naples, whereas I123cdP was not detected in mussels from Kori. ANCPL in mussels from Kori was much higher than that in Naples.

Among the PAH contents analyzed in Yaquina Bay[8)], the concentration of PHEN, FLRTH and PYR were much higher than those in Kori, Korea. While BbF and BghiP in Kori, Korea showed a little higher concentration than those in Yaquina Bay.

In summary, it seems that PAH contents in mussels in Kori, Korea showed similar as somewhat polluted areas of other countries, however, we can not ascertain that the PAH concentration in organisms in Kori might attain to the serious level in the present time. More data on PAH concentration in marine environment in the East Sea area need to be accumulated in near future.

3.3 The sources of PAHs

Pyrogenic or combustion-derived PAH assemblages are relatively enriched in three to five-ring PAH compounds(3～5 ring), whereas uncombusted fossil fuels are highly enriched in the two to three-ring PAHs(2～3 ring)[1)]. As shown in table 3, most PAH of 2～3 ring were much dominant than those of 3～5 ring in mussels in the study area, i.e. the high molecular PAHs were less dominant(Fig. 4).

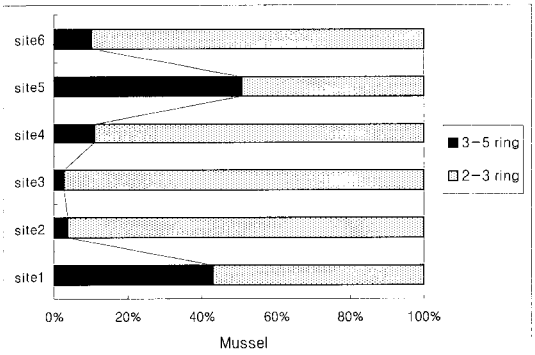

Fig. 4. The ratio of 2～3ring/3～5ring of PAHs in mussels in the study area.

Phenanthrenes compound may be of petrogenic, or diagenetic in origin, naphthalene compounds are characteristic of fresh crude oil, therefore, the

ratio of naphthalene to phenanthrene is particularly diagnostic for inputs of fresh petroleum. The N/P (Naphthalene/Phenanthrene) ratio is much greater than 1.0 for most petroleums and decreases up to below 0.2 in clean sediments[14]. These ratios are useful in defining the hydrocarbon composition of the marine organisms and sediments, and in distinguishing the relative importance of petroleum-derived(petrogenic) hydrocarbons versus biologically derived(biogenic) or combustion-derived (pyrogenic) hydrocarbons.

In this study, the N/P ratio ranged from 2.8 to 825(mean 147) in mussels except at site 6. I suggest that the PAHs in mussels and in the study area is mostly fresh petroleum-derived. Also the relative abundance of two to three-ring PAHs in mussels ranged from 58 % to 90 % of the total(Fig. 4). This confirms the idea that the major source of PAHs in the study area is not combustion-derived. Rather, the major source of PAHs in the study area could be domestic and industrial effluents containing uncombusted fossil fuels, and/or engine or fuel oil spilled from the vessels navigating.

I suggest that seasonal variations of PAH concentrations in mussels, sediment, and seawater as well as other marine organisms in the study area need to be investigated in the future in order to support the above conclusion.

4. Conclusions

NPTHL and ANCPL were dominant PAH compounds in mussels living in the intertidal zone of Kori, Korea, and DahA, BbF, BaP were the next dominant PAH group in mussels in the study area.

It seems that PAH contents in mussels in Kori, Korea, showed similar as somewhat polluted areas of other countries, however, I can not ascertain that the PAH concentration in organisms in Kori might attain to a serious level in the present time. More data on PAH concentration in marine environment in the East Sea area need to be accumulated in near future.

According to N/P(Naphthalene/Phenanthrene) ratio in mussels in the study area, and the ratio of 2~3 ring to 3~5 ring PAH in mussels, we expect that the major sources of PAHs in this study area is rather fresh petroleum-derived. Therefore domestic and industrial effluents containing uncombusted petroleum and fresh fossil fuels from ships navigating the study area could directly affect the PAH contamination in the study area.

Acknowledgement

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